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Original Article

Increased activity of lipoprotein-associated phospholipase A_2 in non-severe asthma

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Lp-PLA2, lipoprotein-associated phospholipase A2; HDL, high density lipoprotein; LDL, low density lipoprotein; GINA, Global Initiative for Asthma; FP, fluticasone proprionate; hsCRP, high-sensitivity C-reactive protein; ETP, endogenous thrombin potential; PAI-1, plasminogen activator inhibitor-1; CLT, clot lysis time

ABSTRACT

Background: Given increased risk of cardiovascular events in asthma we hypothesized that lipoprotein-associated phospholipase A_2 (Lp-PLA_2), an enzyme involved in atherosclerosis, is associated with proinflammatory and prothrombotic blood alterations in this disease.
Methods: In 164 adult asthmatics (63 with severe asthma) we measured plasma Lp-PLA_2 activity using the PLAC test. We determined its relations to inflammation and prothrombotic blood alterations.
Results: In asthma, Lp-PLA_2 was inversely related to the age ($\beta = -0.1$ [−0.18 to −0.02]) and was lower in women (n = 122 [74%], 205 [182–242] vs. 243 [203–262] nmol/min/ml, p = 0.001). Interestingly, Lp-PLA_2 correlated negatively with the asthma severity score ($\beta = -0.15$ [−0.23 to −0.07]), being 10.3% higher in those with non-severe (mild or moderate) asthma (n = 101, 62%) as compared to the severe disease subtype (224 [191–261] vs. 203 [181–229], p = 0.006 after adjustment for potential confounders). Lp-PLA_2 activity was positively related to the levels of low-density lipoprotein ($\beta = 0.1$ [0.02 −0.18]), triglycerides ($\beta = 0.11$ [0.03–0.19]) and glucose ($\beta = 0.1$ [0.02–0.18]) and inversely to the tumor necrosis factor α ($\beta = -0.27$ [−0.35 to −0.2]), high sensitivity C-reactive protein ($\beta = -0.1$ [−0.19 to −0.02]) and fibrinogen ($\beta = -0.12$ [−0.21 to −0.03]), as well as prothrombin ($\beta = -0.16$ [−0.24 to −0.08]), and parameters describing thrombin generation potential, such as endogenous thrombin potential ($\beta = -0.14$ [−0.21 to −0.06]) and peak thrombin generated ($\beta = -0.2$ [−0.28 to −0.12]).
Conclusions: Elevated Lp-PLA_2 activity in non-severe asthmatics suggests increased atherosclerotic risk in this group. Lower Lp-PLA_2 activity accompanied by its inverse relationship to inflammatory or prothrombotic blood biomarkers observed in turn in severe asthmatics might be related to the pathogenesis of more severe asthma phenotype.

Introduction

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a member of phospholipase A_2 superfamily. The exact biological function of this enzyme in humans has not been clarified yet. It is still controversial with seemingly contradictory anti- or pro-atherogenic functions being proposed.[1] Also the evaluation of the distribution of Lp-PLA_2 in the lipid fractions emphasized the dual role of that enzyme in the vessel inflammatory process. The circulating Lp-PLA_2 enzyme in 20% is related to the high density lipoprotein (HDL) and contributes to the reduction of atherosclerosis, while in 80% is associated with atherogenic low-density lipoprotein (LDL).[1–3]

The secreted isoform of LP-PLA_2 was first identified on the basis of its ability to degrade platelet-activating factor (PAF), prothrombotic and proinflammatory mediator, hence its formed name was PAF-acetylhydrolase (PAF-AH).[1] This enzyme is highly

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expressed by macrophages, T-lymphocytes, monocytes, and mast cells in atherosclerotic lesions, particularly within the necrotic core and fibrotic cap of rupture-prone plaques.[4,5] It has been demonstrated that Lp-PLA_2 hydrolyzes oxidized phospholipids and yields pro-inflammatory and pro-atherogenic products implicated in endothelial dysfunction with the subsequent atherosclerosis progression.[6,7] Recent studies have shown that increased Lp-PLA_2 activity or mass is an independent risk marker for cardiovascular disease (CVD), including coronary heart disease (CHD), and ischemic stroke.[2,5] Moreover, its activity is highly specific for inflammation associated with atherosclerosis, and is not likely to be falsely elevated from rheumatic diseases, infections or obesity, so it might be used as an endothelium damage biomarker in diseases associated with low-grade systemic inflammation.[2]

Asthma is a chronic inflammatory condition of the airways, characterized by bronchial reversible obstruction and hyperresponsiveness.[8] Specific patterns of clinical or pathophysiological features of asthma have been identified, leading to recognition of asthma phenotypes.[9] Recently, epidemiologic studies have demonstrated that this disease is also associated with an increased risk of cardiovascular[10–13] and thromboembolic events.[14–16] The mechanisms underlying this phenomenon are largely unknown. In 2016 we demonstrated that asthma is related to the enhanced plasma thrombin formation, impaired clot lysis, and blood platelet activation,[17] which is in line with the study of Sneeboer *et al.*[18] We also observed that a hypercoagulable state in this disease was related to increased levels of inflammatory cytokines, including interleukin (IL)-6 and tumor necrosis factor (TNF)α in circulating blood.[19] Moreover, some prothrombotic plasma alterations have been shown to lead to the higher exacerbation rate among asthmatics.[20] Interestingly, documented prothrombotic blood abnormalities in asthma have been strongly associated with higher cellular fibronectin (cFN) levels, a marker of vascular injury,[21] which was positively correlated with inflammatory parameters, thus it was not clear whether increased cFN reflects solely endothelial damage or rather low-grade systemic inflammatory state. In turn, atherosclerosis and asthma may share similar pathophysiological mechanisms including tissue and vascular injury and inflammation.[22,23] Previously, impaired flow mediated dilation of the brachial artery,[24] and increased thickness of the carotid and femoral intima-media complex[25] have been demonstrated in asthmatic patients, suggesting endothelial dysfunction and subclinical atherosclerosis. To date, only scarce papers have addressed issue of Lp-PLA_2 in asthma. Triggiani *et al.*[26] has found lower Lp-PLA_2 activity in bronchoalveolar lavage fluid of intermittent asthmatics in comparison to healthy controls. Tsukioka *et al.*[27] and Stafforini *et al.*[28] have reported lower circulating Lp-PLA_2 activity in stable asthma patients with no clear association to the disease severity. These observations might contradict our hypothesis about accelerated atherosclerosis in asthma, however they were performed many years ago, and mainly on Japanese population. Given complex associations between inflammation, prothrombotic state, atherosclerosis and endothelial injury in asthma that have been reported recently,[10–13] we sought to evaluate plasma activity of Lp-PLA_2 in asthmatics and assess their relations to prothrombotic blood alterations and inflammatory markers.

Methods

Subjects studied

We enrolled 164 white adult subjects with clinically stable asthma (63 [38.4%] patients with severe asthma) recruited at the outpatient clinic in Cracow, Poland. The patient characteristics, together with inclusion and exclusion criteria, have been described in our previous paper in detail.[17] Briefly, diagnosis of asthma was established based on recurrent respiratory symptoms in medical history, including chest tightness and cough, wheeze, and shortness of breath, as well as currently or historically documented post bronchodilator increase in forced expiratory volume in 1 s (FEV_1) of at least 12% and 200 ml from the baseline.[8] All asthma medications at screening, with the exception of omalizumab, were allowed, including oral corticosteroids at a daily dose equivalent to $\leq$8 mg of methylprednisolone. Asthma patients could not be exacerbated during the 6 months before the enrollment. Moreover, active skin lesions at enrollment were an exclusion criterion. Severity of asthma was categorized according to the Global Initiative for Asthma (GINA) guidelines.[8] "Mild" asthma was defined as mild persistent disease, well-controlled on inhaled short-acting β_2-agonists on demand or receiving low daily dose of inhaled corticosteroids (ICS) (<250 μg of fluticasone propionate [FP] [dry powder inhaler] or equivalent). "Moderate" asthma was defined as mild persistent disease treated with low dose of ICS combined with long-acting β_2-agonists, or medium dose of ICS (250–500 μg of FP or equivalent). "Severe" asthma was defined as asthma that requires high dose of ICS (>500 μg of FP or equivalent) with long-acting β_2-agonist to prevent it from becoming uncontrolled or asthma that remains uncontrolled despite this treatment. Non-severe asthma was defined as mild or moderate asthma, as characterized above. Spirometry was performed using a Jaeger, Master Screen spirometer in all enrolled subjects according to the American Thoracic Society standards.[29] If basal spirometry showed bronchial obstruction, a bronchial reversibility test with 400 μg of albuterol was performed and parameters of the second spirometry were further analyzed.

The study has been approved by the Ethics Committee of the Jagiellonian University (approval number: KBET/43/B/2014). Informed consent was obtained from all participants included in the study and patient anonymity was preserved using methods approved by the Ethics Committee.

Laboratory investigations

Subjects were fasted overnight and blood was sampled between 08.00 and 10.00 A.M. using minimal stasis. Basic laboratory tests, including liver enzymes, creatinine level, lipid profile, glucose, fibrinogen, as well as blood cell and platelet count were analyzed by routine laboratory techniques. Immunoglobulin E (IgE) and high sensitivity C-reactive protein (hsCRP) were measured as previously described.[17] Blood samples were drawn into tubes containing sodium citrate and serum separation tubes, centrifuged at 2000 g for 20 min at room temperature, within 2 h from sampling. The supernatant was frozen in aliquots and stored at −70 °C until further analysis.

To assess thrombin generation, the Calibrated Automated Thrombogram (CAT) was used as previously described.[17] The maximum concentration of thrombin generated in this assay is described as the "thrombin peak", while the area under the curve of thrombin formation represents the "endogenous thrombin potential" (ETP). The CAT assay was obtained using commercially available reagents (Thrombinoscope, BV, Maastricht, Netherlands) as described previously.[30]

Plasma prothrombin and antithrombin activities were measured using the ACL TOP 500 CTS analyzer and appropriate reagents (Instrumentation Laboratory, Bedford, MA, USA).[17]

Commercially available immunoenzymatic assays were used to analyze TNFα and IL-6,[19] P-selectin (all, R&D Systems, Minneapolis, MN, USA),[17] and plasminogen activator inhibitor-1 (PAI-1) (American Diagnostica, Stamford, CT, USA).[17] Plasminogen and antiplasmin activities were obtained in chromogenic assays (STA

Stachrom plasminogen and STA Stachrom α2-antiplasmin, Diagnostica Stago, Asnieres, France).[17]

Clot Lysis Time (CLT) was determined to evaluate plasma global fibrinolytic capacity as previously described.[31]

The Lp-PLA_2 activity in plasma was measured by the PLAC Test (Diazyme Laboratories, San Diego, CA, USA) according to the manufacturer's instruction. In this assay Lp-PLA_2 hydrolyzes the sn-2 position of 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine (the reaction substrate), producing a colored reaction product, 4-nitrophenol. The rate of formation of 4-nitrophenol is assessed spectrophotometrically and the Lp-PLA_2 activity is calculated from the rate of change in absorbance based on a standard curve fit generated from five Lp-PLA_2 calibrators. In 50 healthy subjects, Lp-PLA_2 activity were from 10 to 120 (median 90) nmol/min/ml (A. Undas unpublished data).

Statistical analysis

Analysis was performed using the STATISTICA 12.5 software package (StatSoft, Inc, Tulsa, OK, USA). Normality was checked by the Shapiro–Wilk W-test. Continuous variables, all non-normal distributed, were reported as medians and quartiles. Categorical variables were given as percentages. Comparisons were made by the Mann–Whitney U test. Categorical variables were compared by the χ^2 test. To adjust for potential confounders, including age, body mass index (BMI), and sex, all non-normal distributed data were log-transformed and a one-way covariance analysis (ANCOVA) was performed, which resulted in an overall p-value. Associations between continuous variables were calculated by univariate linear regressions with adjustment for age, sex, and BMI. Independent determinants of Lp-PLA_2 were established in multivariable linear regression models, built by a forward stepwise selection procedure, verified by Snedecor's statistics with $F > 1$ and without residues autocorrelation (checked by Durbin–Watson's statistics). The R^2 was used as a measure of the variance.

P-values <0.05 were considered statistically significant.

Results

Patient characteristics

Detailed characteristics of the studied patients have been demonstrated in our previous publication.[17] Briefly, we analyzed 63 (38.4%) severe asthmatics, as well as 45 (27.4%) subjects with moderate and 56 (34.1%) with mild subtype of the disease. Among all 164 asthma patients 42 (25.6%) were males. The 144 (87.8%) subjects received inhaled corticosteroids, 26 (15.8%) theophyllin, 124 (75.6%) long-acting β_2-agonists, 40 (24.4%) montelukast, and 34 (20.7%) oral corticosteroids.

Patients with severe asthma were older (Table 1). They had also higher number of white blood cells, as well as increased thrombin formation parameters as compared to non-severe (mild and moderate) asthmatics.

Table 1
A summary of clinical and laboratory variables in the subjects studied.

	Non-severe asthma n = 101 (61.6%)	Severe asthma n = 63 (38.4%)	p-value
Clinical data			
Age, years	48 (36–59)	56 (50–64)	0.007
Male gender, n (%)	25 (25)	17 (27)	0.75
Body mass index (kg/m^2)	26.5 (24.1–29.6)	26.7 (23.5–29.7)	0.96
Current smoking, n (%)	17 (17)	14 (22)	0.39
Arterial hypertension, n (%)	33 (33)	24 (38)	0.48
Diabetes mellitus, n (%)	6 (6)	7 (11)	0.23
Hypercholesterolemia, n (%)	50 (50)	38 (60)	0.18
Allergic rhinitis, n (%)	77 (76)	47 (75)	0.81
Atopic dermatitis, n (%)	10 (10)	8 (13)	0.58
Blood platelets (10^3/μl)	215 (187–243)	214 (189–265)	0.33
Red blood cells (10^6/μl)	4.73 (4.49–5.05)	4.8 (4.57–5.08)	0.47
Hematocrit (%)	41.7 (40–44.2)	42.2 (40.8–44.1)	0.3
White blood cells (10^3/μl)	6.35 (5.18–7.33)	7.13 (5.79–8.64)	0.002
Total cholesterol (mmol/l)	5 (4.3–5.6)	5.2 (4.5–6.2)	0.08
Triglycerides (mmol/l)	1.12 (0.9–1.7)	1.22 (1.01–1.77)	0.3
Glucose (mmol/l)	5 (4.6–5.3)	4.9 (4.5–5.3)	0.84
Fibrinogen (g/l)	3.6 (2.9–3.9)	3.6 (3.2–4.1)	0.21
Prothrombin (%)	109 (99–117)	107 (99–119)	0.88
Antithrombin (%)	102 (93–114)	101 (90–110)	0.2
high-sensitivity C-reactive protein (mg/l)	1.21 (0.46–2.35)	1.3 (0.62–3.49)	0.14
Interleukin-6 (pg/ml)	4.55 (3.58–5.68)	4.55 (3.58–5.98)	0.82
Tumor necrosis factor α (pg/ml)	4.05 (3.02–4.7)	3.76 (2.91–4.69)	0.72
P-selectin (ng/ml)	22.38 (16.44–33.02)	23.94 (15.04–38)	0.73
Endothelial injury			
Lipoprotein-associated phospholipase A_2 (nmol/min/ml)	224 (191–261)	203 (181–229)	0.009
Thrombin generation and dynamic of thrombin formation			
Thrombin peak (nmol/l)	279.6 (242.8–313)	287.5 (252.8–347)	0.13
Endogenous thrombin potential (nmol/l thrombin x min)	1489 (1304–1620)	1545 (1368–1811)	0.04
Lag-time (min)	2.33 (2–2.33)	2.33 (2–2.67)	0.3
Time to thrombin peak (min)	5 (4.67–6)	5 (4.33–6.33)	0.72
Fibrinolysis			
Clot lysis time (min)	93.4 (71.7–113.5)	102.9 (68.6–124.4)	0.2
Plasminogen (%)	105 (94–118)	107 (96–120)	0.58
Antiplasmin (%)	104 (98–116)	101 (95–110)	0.1
Plasminogen activator inhibitor-1 (ng/ml)	28.4 (21.7–39.4)	30.09 (22.02–43.88)	0.33

Categorical variables are presented as numbers (percentages), continuous variables as median and interquartile range.
n, number of subjects.

Lipoprotein-associated phospholipase A_2

In asthma, Lp-PLA_2 activity was inversely related to the age ($\beta = -0.1$ [−0.18 to −0.02]) and was lower in women than in men (205 [182–242] vs. 243 [203–262] nmol/min/ml, $p = 0.001$). Interestingly, this enzyme activity was inversely related to the asthma severity score according to the GINA ($\beta = -0.15$ [−0.23 to −0.07]). Moreover, non-severe (mild and moderate) asthma was characterized by 10.3% higher Lp-PLA_2 activity than severe type of the disease ($p = 0.006$ after adjustment for potential confounders) (Table 1, Fig. 1). We found no difference in Lp-PLA_2 activity in relation to allergic rhinitis, history of atopic dermatitis, and other internal medicine comorbidities. Also medications used, including oral corticosteroids, had no impact on Lp-PLA_2 activity.

Weak but significant positive correlations were demonstrated between Lp-PLA_2 activity and blood eosinophilia ($\beta = 0.08$ [0.01–0.16]), LDL ($\beta = 0.1$ [0.02–0.18]), triglycerides ($\beta = 0.11$ [0.03–0.19]) and glucose ($\beta = 0.1$ [0.02–0.18]) levels. Interestingly, Lp-PLA_2 was inversely related to the inflammatory markers, such as TNFα ($\beta = -0.27$ [−0.35 to −0.2]), hsCRP ($\beta = -0.1$ [−0.19 to −0.02]) and fibrinogen ($\beta = -0.12$ [−0.21 to −0.03]), but not to IL-6. Moreover, we documented negative relationships of Lp-PLA_2 activity with prothrombin ($\beta = -0.16$ [−0.24 to −0.08]) and parameters describing quantitatively thrombin generation, such as endogenous thrombin potential ($\beta = -0.14$ [−0.21 to −0.06]) and peak thrombin generated ($\beta = -0.2$ [−0.28 to −0.12]). Conversely, relation of LP-PLA_2 activity to the traits assessing dynamic of thrombin formation, including time to the beginning of thrombin formation (lagtime) and time to the thrombin peak, remained positive ($\beta = 0.1$ [0.02–0.18] and $\beta = 0.15$ [0.07–0.23], respectively).

Table 2 shows a multivariable linear regression model with independent determinants of Lp-PLA_2 activity in severe asthma. The Lp-PLA_2 activity was predicted by hematocrit, antithrombin, glucose and PAI-1 levels, with negative impact of prothrombin and TNFα (Table 2). Interestingly, in severe asthma Lp-PLA_2 activity was inversely related to the FEV_1 (Table 2).

In non-severe asthma the Lp-PLA_2 activity was also determined by hematocrit, followed by triglycerides, P-selectin, and peak thrombin generated, with negative contribution of TNFα, fibrinogen, white blood cell count, and antiplasmin (Table 3).

Discussion

In the present study we for the first time have shown that Lp-PLA_2 activity in asthma patients depends on asthma severity, being higher in those with non-severe asthma. Previously published reports have documented lower Lp-PLA_2 activity in bronchoalveolar lavage fluid and blood of asthmatic subjects[26–28] in comparison to healthy controls, with no difference regarding asthma severity. However, in these studies published in 1990s Lp-PLA_2 activity was determined using different methods based on radioimmunoassays. Moreover, they were performed mostly on Japanese population and might mirror the characteristics of this population.

Clinical relevance of Lp-PLA_2 in humans remains unclear. It has been previously shown that circulating concentrations or activity of Lp-PLA_2 are higher in men than in women, which is in line with our results.[1] Surprisingly, we found that Lp-PLA_2 in asthma correlates inversely with age. Regarding laboratory variables, Corsetti *et al.*[32] studied the association between Lp-PLA_2 activity and clusters of lipid measurements, hemostatic biomarkers, inflammatory and glycemic control measures in post-infarction patients. They found positive relationships of Lp-PLA_2 to total cholesterol, apolipoprotein B, triglycerides and inverse with HDL level. The fact that Lp-PLA_2 in this study was associated with atherogenic phenotype was not surprising, given that 80% of the enzyme is bound to apolipoprotein B. In our study we also demonstrated a weak but positive relationship between Lp-PLA_2 and LDL or triglycerides in asthmatics. However, a negative correlation between Lp-PLA_2 activity and inflammatory markers is an interesting finding, suggesting that asthma inflammation disturbs known from the CVD group relationship and might decrease Lp-PLA_2 activity measurements with a false negative result of PLAC test,

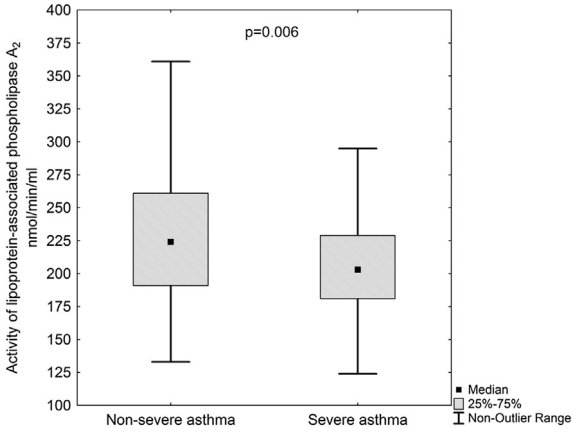

Fig 1. Lp-PLA2 activity (nmol/min/ml) according to asthma severity.

Table 2
Independent determinants of lipoprotein-associated phospholipase A_2 in severe asthma.

Severe asthma, n = 63 (38.4%)	β	95% CI
Age (years)	−0.33	−0.42 to −0.24
FEV_1 (% of predicted value)	−0.3	−0.37 to −0.22
Hematocrit (%)	0.26	0.18 to 0.34
Glucose (mmol/l)	0.22	0.14 to 0.29
Prothrombin (%)	−0.27	−0.34 to −0.2
Antithrombin (%)	0.26	0.16 to 0.34
Tumor necrosis factor α (pg/ml)	−0.26	−0.35 to −0.17
Plasminogen activator inhibitor-1 (ng/ml)	0.08	0.004 to 0.16
	$F = 8.8$, $R^2 = 0.49$, $p < 0.001$	

The resulting standardized regression coefficient (β) with 95% confidence intervals (95%CI) for a factor (independent variable) indicates the increase/decrease in standard deviations (SDs) of dependent variable, when that particular factor increases with 1 SD and all other variables in the model are unchanged.
n, number of subjects.

Table 3
Independent determinants of lipoprotein-associated phospholipase A_2 in non-severe asthma, defined as a mild or moderate type of the disease.

Non-severe asthma, n = 101 (61.6%)	β	95% CI
Hematocrit (%)	0.13	0.05 to 0.2
White blood cells (10^3/μl)	−0.26	−0.33 to −0.19
Tumor necrosis factor α (pg/ml)	−0.3	−0.37 to −0.24
Alanine aminotransferase (IU/l)	0.11	0.04 to 0.17
P-selectin (ng/ml)	0.2	0.13 to 0.26
Triglycerides (mmol/l)	0.11	0.04 to 0.18
Fibrinogen (g/l)	−0.09	−0.16 to −0.03
Antiplasmin (%)	−0.07	−0.14 to −0.01
Thrombin peak (nmol/l)	0.1	0.03–0.18
	$F = 6.7$, $R^2 = 0.24$, $p < 0.001$	

The resulting standardized regression coefficient (β) with 95% confidence intervals (95% CI) for a factor (independent variable) indicates the increase/decrease in standard deviations (SDs) of dependent variable, when that particular factor increases with 1 SD and all other variables in the model are unchanged.
n, number of subjects.

recommended by the American Heart Association for the CVD risk stratification in selected subjects.[2] That difference with lower enzyme activity might be also related to the asthmatic medications, particularly inhaled glucocorticosteroids, which were used in high doses in all severe asthma patients. Interestingly, Corsetti *et al.*[32] documented also a nonsignificant trend towards lower Lp-PLA_2 activity at elevated CRP levels.

On the other hand, it has been shown that Lp-PLA_2 inactivates PAF.[3] Higher PAF levels have been reported in asthmatics.[33,34] PAF might be responsible for airway hyperactivity or persisting airway inflammation, including activation of eosinophils and neutrophils to release granule constituents and to generate superoxide anions.[34] Therefore, higher PAF resulting from lower Lp-PLA_2 activity could be related to the further increase in inflammation, explaining at least partially an inverse correlation of this enzyme activity with inflammatory markers observed in our study. Henig *et al.*[35] have shown that intravenous treatment with recombinant human Lp-PLA_2 did not significantly reduce either the early or late asthmatic response in mild atopic asthma subjects, although recombinant Lp-PLA_2 inhibited airway inflammation and hyperreactivity in a murine asthma model in mice.[36] Our results would suggest that such therapy might be helpful rather in severe, than mild asthma, since those subjects are characterized by lower Lp-PLA_2 activity. Importantly, we found no relation between the use of systemic corticosteroids and Lp-PLA_2 activity, which is in line with previous results of Stafforini *et al.*[28] Thus, lower Lp-PLA_2 activity in severe asthmatics is likely relating to the pathology of a more severe asthma phenotype.

It is still unclear however, whether higher plasma activity of Lp-PLA_2 observed in non-severe asthmatics is related to the pathology of less severe disease course or accelerated atherosclerosis.

Study limitations

The study group was relatively small. Subgroup analysis should be interpreted with caution. We determined each variable at a single time point, thus we cannot exclude changes of the variables analyzed over time. We did not determine other potential modulators of asthma severity, e.g. missense Val279Phe Lp-PLA_2 gene mutation, which as it has been demonstrated in Japanese population might be more predominant in more severe asthma phenotype.[28] We did not study PAF levels, an important substrate for Lp-PLA_2. We also did not determine other phospholipase A_2 isozymes. Statistical associations reported here might not necessarily indicate cause-effect relationships. In vitro models are needed to elucidate the role of Lp-PLA_2 in asthma pathology, particularly of atherosclerosis. Finally, clinical relevance of increased blood Lp-PLA_2 activity in non-severe asthmatics in relation to asthma course, progression of bronchial obstruction and vascular outcomes remains to be established.

Conclusions

The Lp-PLA_2 activity is higher in subjects with non-severe asthma, suggesting increased atherosclerotic risk in this group. In turn, lower Lp-PLA_2 activity in severe asthmatics, with inverse relationship of Lp-PLA_2 to inflammatory and prothrombotic blood biomarkers, might relate to the pathogenesis of more severe asthma phenotype, although large observational studies are needed to verify this hypothesis.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

SBS and AU designed the study. PK, LM, AC and DPP contributed to the data collection. Statistical analysis was conducted by LZ. PK, SBS and AU interpreted the results and wrote the manuscript. All authors read and approved the final manuscript.

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